



From Retrospective Assessment to Prospective Decisions in Natural Product Isolation

Johansen, Kenneth

Publication date:
2011

Document version
Early version, also known as pre-print

Citation for published version (APA):
Johansen, K. (2011). *From Retrospective Assessment to Prospective Decisions in Natural Product Isolation*.
Poster session presented at SMASH 2011 NMR Conference, Chamonix, France.

From Retrospective Assessment to Prospective Decisions in Natural Product Isolation

Kenneth T. Johansen^{1*} and Jerzy W. Jaroszewski¹

¹Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100

*E-mail: kj@farma.ku.dk

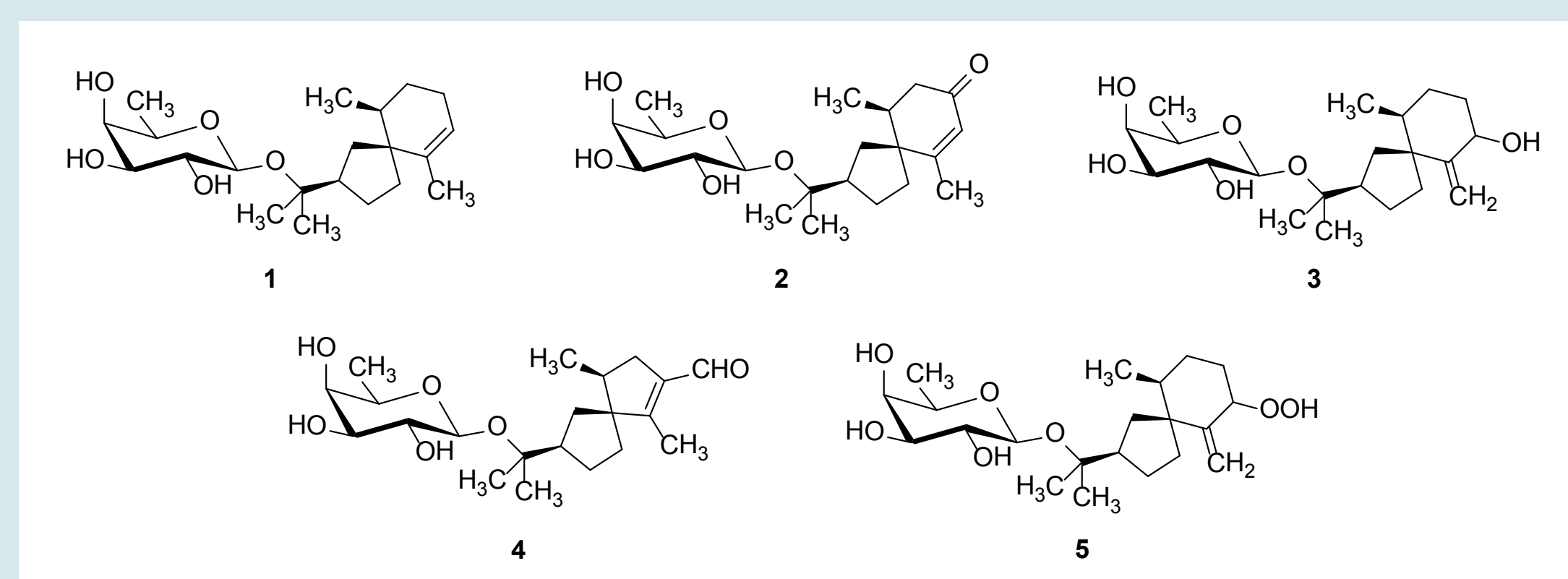
Introduction

Repetitive isolation of known or even readily available natural products is one of the main factors limiting productivity of natural products research. Dereplication, i.e., recognition and exclusion from further isolation efforts of extract constituents that have already been studied or are otherwise unwanted is therefore a cornerstone of lead discovery programs based on natural products. With advances in miniaturization and cryogenically cooled probe-designs, the structurally information-rich NMR-technique has, when coupled to a HPLC-system, become a viable alternative or supplement to the commonly employed HPLC-PDA-MS dereplication strategy.

The focus of this work is to show how NMR data, obtained through analytical scale HPLC separation, enables us to focus our effort at isolating only compounds of interest.

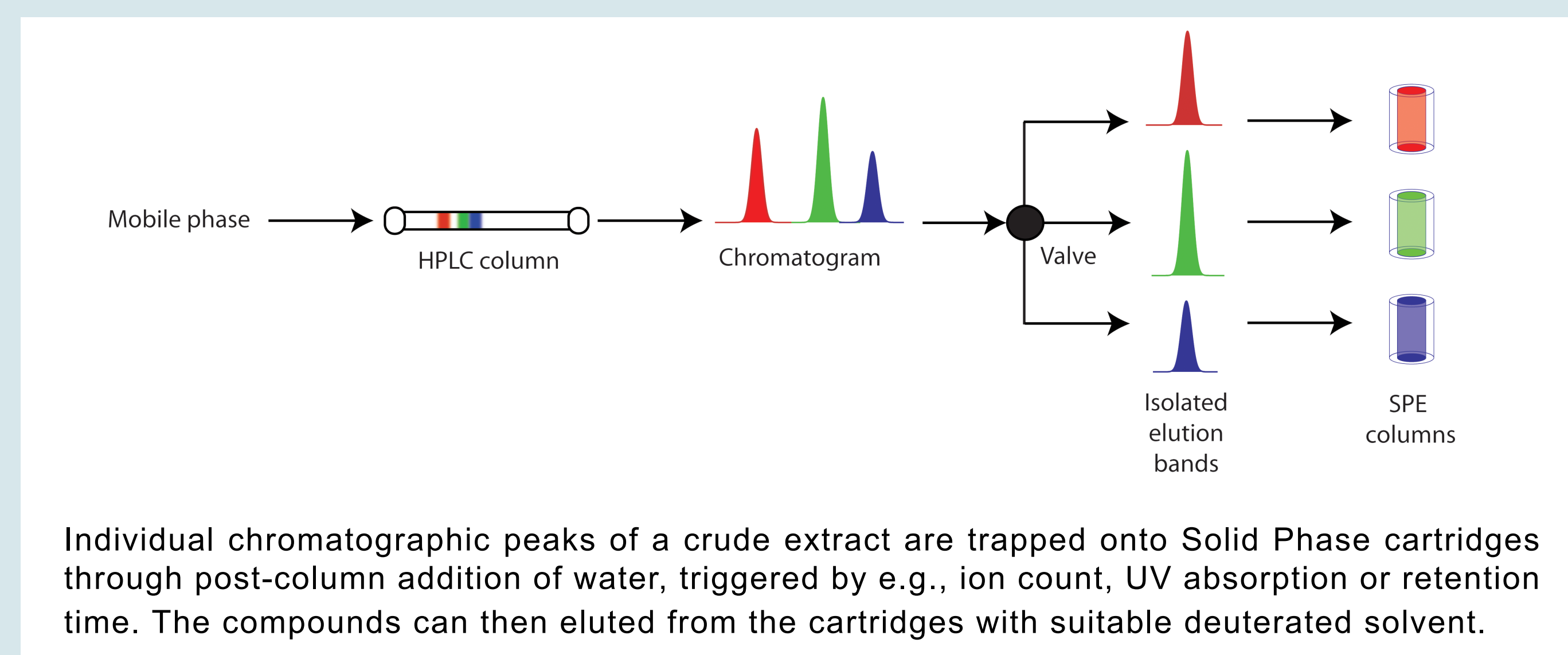
The compounds of interest

In the present work, we show how the structural information obtained from 30 chromatographic peaks through HPLC-PDA-HRMS-SPE-NMR led to a rapid, targeted isolation of 3 unusual spiro[4.5]decane derived (**1-3**) and 1 spiro[4.4]nonane derived (**4**) sesquiterpenoids from the plant *Carthamus oxycantha*, while tedious isolation of unwanted metabolites was omitted. For the sake of comparison, a traditional approach to the fractionation was also performed. Furthermore, the mild conditions of the hyphenated analysis allowed us to isolate a hydroperoxide (**5**), which we were not able to isolate in neither the traditional approach, nor through the targeted isolation.



The HPLC-PDA-HRMS-SPE-NMR technique

Advances within the area of NMR hyphenation have led to implementation of an on-line solid phase extraction (SPE) step between the outlet of HPLC column and the NMR spectrometer. By lowering the elutropic strength of the eluent through addition of water, trapping of metabolites onto SPE cartridges can be triggered by, e.g., ion count (mass detection), absorption (UV detection), or retention time. After automated drying under N₂, the metabolites are transferred with deuterated solvents to either a flow-probe or into NMR tubes in automation.



One of the numerous advantages of the HPLC-SPE-NMR setup over other hyphenated NMR techniques [1][2][3] is the increased sensitivity obtainable by analyte focusing and repeated trappings. This enables us to obtain analytical scale chromatographic separation comparable to HPLC-MS methods.

When combined with a cryogenically cooled 1.7 mm probe installed in our 600 MHz magnet with sensitivity in the ng-range [4], it is possible to obtain structural information about the majority of metabolites in a plant through analysis of less than 10 mg defatted crude extract.

Traditional analysis of *Carthamus oxycantha*

9.5 g crude extract of *Carthamus oxycantha* was fractionated by VLC and preparative HPLC during a total of ca. 3 months.

This led to isolation of spiro-compounds **1-4**, which all showed sign of partial acidic cleavage of the glycosidic bond during sample work-up. Furthermore, a range of 'nuisance' compounds, were also isolated, including: Vanillic acid, different caffeic acid derivatives, flavonol and chalcone glycosides, flavonoids, polyphenols, and fatty acids.

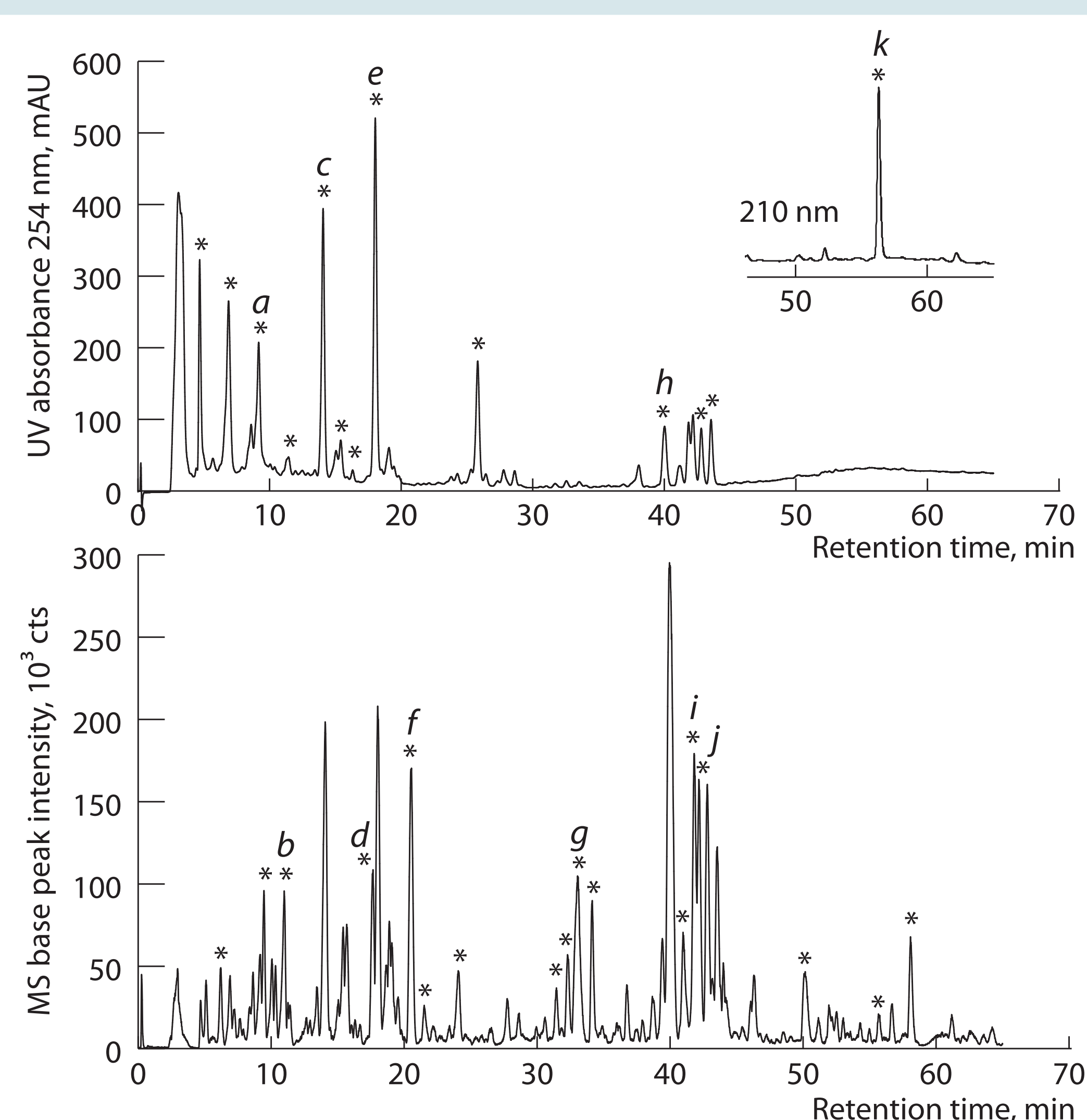
References

- [1] Kesting JR, Johansen KT, Jaroszewski JW in Biomolecular NMR Spectroscopy, Chapter 19, IOS Press, 2011; 3
- [2] Exarchou V et al. *Current Trends in Analysis and Characterisation* 2006: 143-155
- [3] Jaroszewski JW, *Planta Med* 2005; 71: 795-82
- [4] Hilton BD, Martin GE, *J. Nat. Prod.* 2010; 73: 1465-69

The HPLC-PDA-HRMS-SPE-NMR experiments

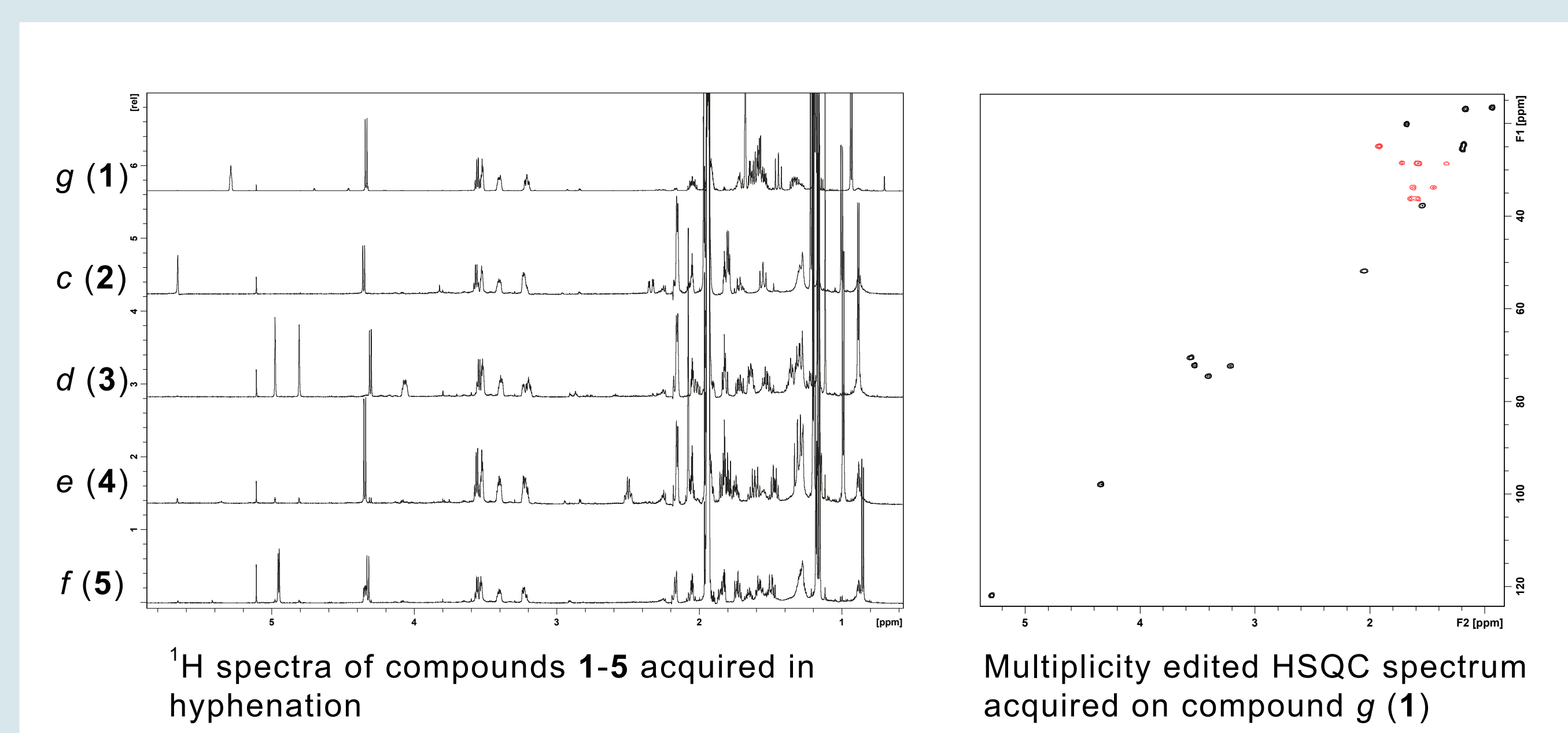
1 mg defatted extract of *Carthamus oxycantha* was separated on a C18 4.6 x 150 mm, 3µm column using a linear acetonitrile gradient in water over 50 min, trapping 30 compounds (marked with asterisks) through 2 cumulative injections. Analysis of ¹H NMR spectra allowed identification of nuisance compound, excluded from further analysis, as well as 11 compounds deemed valuable from peaks a-k. These compounds were subjected to complete analysis by 2D NMR after 8 cumulative trappings (in total 4 mg extract).

2D NMR experiments of the 11 samples led to elucidation of a series of spiro-compounds **1-5** as well as several unsaturated and oxygenated fatty acids.



Crude extract of *Carthamus oxycantha* analysed by HPLC-PDA-HRMS-SPE-NMR. Initial analysis of compounds marked with asterisks (*) was followed by full structure elucidation by 2D NMR experiments of compounds a-k.

Trapping of the compounds were based on a combination of MS and UV chromatograms



Conclusion and perspectives

This study shows how hyphenated NMR can provide full structures of components of a natural product extract, being useful as a fast, reliable and robust dereplication tool. The procedure not only let us exclude trivial compounds from further isolation efforts, but furthermore enabled us to identify a series of novel spiro-compounds - a task that would be impossible with traditional dereplication tools such as HPLC-MS.

Structural information obtained through hyphenation furthermore let us adjust the isolation procedure specifically to target the acid-labile spiro compounds.

This work demonstrates that hyphenated HPLC-SPE-NMR technique, aided by cryogenic microprobe, is an effective dereplication technique that enables complete structural elucidation of extract components fast and on a micro-scale. Based on the results obtained by this technique, optimal targeted isolation procedures can be designed for selected compounds. This approach saves time, labour and consumables compared to classical isolation procedures and traditional dereplication based on MS alone.

Acknowledgements

The authors would like to thank Bruker Biospin GmbH and the Drug Research Academy for co-financing the PhD fellowship to Kenneth T. Johansen.